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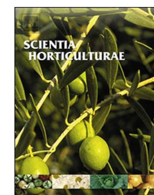

A system for large scale production of chrysanthemum using microponics with the supplement of silver nanoparticles under light-emitting diodes

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A R T I C L E I N F O

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A B S T R A C T

A microponic culture system, combining micro-propagation and hydroponics, could reduce the drawbacks of micro-propagation system such as being prone to contamination, being resource-intensive and require large areas, etc. In this study, chrysanthemum shoots (3 cm in length) were cultured in a microponic system and micro-propagation system. The growth of shoots cultured on half-strength sugar-free liquid MS medium supplemented with 7.5 ppm silver nanoparticles under 70% red LED combined with 30% blue LED were the highest among tested concentrations. Results of qualitative and quantitative tests of microbial contents in the microponic culture by 4 testing methods indicated that at concentration of 7.5 ppm silver nanoparticles effectively reduces microbial counts of 8 tested bacteria (*Corynebacterium* sp., *Enterobacter* sp., *Arthrobacter* sp., *Agrobacterium* sp., *Xanthomonas* sp., *Pseudomonas* sp., *Bacillus* sp. and *Micrococcus* sp.) and 3 fungi (*Aspergillus* sp., *Fusarium* sp. and *Alterneria* sp.). After 12 weeks at the nursery stage, the chrysanthemum plants derived from microponics began to bear flower-buds. Chrysanthemums produced by the microponic system started flowering after 15 weeks, which was 1 week earlier than those produced by *in vitro* micropropagation. Taken together, a microponic system was developed and tested successfully. The shoots derived from microponic culture could flower normally at 1-week earlier than the micropropagation-derived ones. The approach was proved to be both cost and time saving.

1. Introduction

Chrysanthemums are a popular flower for pots and cut flowers globally (Zhang et al., 2013) with billions of branches being sold each year, and is available in a wide range of colors (white, yellow, blue, red, purple, pink, etc.) as well as a range of shapes and sizes of flowers. Chrysanthemum is one of the most major cut flower crops in Vietnam, occupying about 25% of the cut flower production area (Linh, 1998).

As production expanded, demand for seedlings also increased. Chrysanthemum is propagated mainly by cuttings because it is simple, economical and able to be carried out under *ex vitro* conditions. However, this method has some limitations such as: a low multiplication coefficient, poor quality of seedlings, etc. Consequently, there is need for a more efficient system for propagation.

Micro-propagation proves to be an effective method for multiplying plants in large scale in a short time. The success of micro-propagation methods depends heavily on the success of acclimatization in nursery conditions. *In vitro*, the growth of plants is controlled while in the acclimatization stage plants are exposed to numerous adverse external factors such as microbial pathogens (mostly fungi and bacteria), temperature fluctuation, low humidity, and poor nutrition which reduce the survival rate of plants significantly (Valero-Aracama et al., 2006; Mathur et al., 2008). In addition, when transferred to *ex vitro* conditions, physiological changes within the plant organs also lead to morphological and anatomical abnormalities. In a general micro-propagation system, stomata of plants do not function properly, roots are weak, and the epidermal layer is thin (Mathur et al., 2008). During this adaptation stage, studies have demonstrated changes in sugar, starch, protein solubility, etc. in oats (Valero-Aracama et al., 2006). Therefore, understanding the physiological and biochemical changes in plants during adaptation is essential to devise measures for improving the survival rate, growth and development of plants in greenhouse.

Till now, silver nanoparticles were proved to possess potential to inhibit microbial contamination (e.g., fungal and bacterial infections)

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in micro-propagation (Sarmast et al., 2011; Sahu et al., 2012) and effects of ethylene (Kaveh et al., 2013), hence, improved *in vitro* plant growth (Savithramma et al., 2012; Syu et al., 2014). However, no study has reported on the inhibition of microbial contamination in growth mediums or in culture and the growth and development of plants in a microponic system.

In recent years, light emitting diodes (LEDs) have become an alternative light source for plant tissue culture. LEDs have improved features, such as smaller mass and volume, longer lifetime and tailored wavelength spectrum compared to conventional fluorescence lamp (FL) (Bula et al., 1991). Furthermore, some studies have shown that LEDs were found to be more suitable for *in vitro* and *ex vitro* plant growth than fluorescent lamps (Dutta Gupta and Jatothu, 2013; Olle and Virsile, 2013). In this study, we also test the effects of LEDs on growth as well as on the quality of seedlings produced by microponics.

Microponic systems are a propagation approach which combines the advantages of micro-propagation and hydroponics. This system was first described by Hahn et al. (1996, 1998, 2000) and it used the nutrient film technique, with a small pump for medium circulation through Rockwool. Culture conditions (temperature, CO_2, humidity, pH and electrical conductivity) were controlled so that the photosynthesis rate, fresh weight, dry weight, leaf size, leaf number, and stomata density could be improved. However, previous studies on this system only evaluated the morphological characteristics of plant growth without further assessment of physiological changes as well as application for commercial production.

2. Materials and methods

2.1. Materials

2.1.1. Plant material

Chrysanthemum morifolium Ramat cv. Jimba shoots (3 cm in length and 2 pairs of leaves) were used as an explant source. These shoots were obtained from a mass of *in vitro* shoots cultured on MS medium (Murashige and Skoog, 1962) with 8 g l^{-1} agar and 30 g l^{-1} sucrose after 45 days of cultured.

2.1.2. Silver nanoparticle solution

Silver nanoparticles (AgNPs) with size of ≤20 nm were set in accordance with the following proportions: $AgNO_3$ 750–1000 ppm, β – chitosan 250–300 ppm, $NaBH_4$: 200 ppm, mole ratio $NaBH_4/AgNO_3$: ¼ and the drip rate of $NaBH_4$ is 10–12 drops per minute (Chau et al., 2008).

2.1.3. Substrate and culture systems

Nylon films (20 cm × 30 cm) were wrapped around a test tube with an outer diameter of 1.5 cm. Then, they were fused and sealed by a heated metal rod (Nhut et al., 2005). Next, the test tube was removed, tube shaped nylon film was cut into short tubes with 2 cm in length. These tubes were used as the substrate in the microponic system. 600 substrates were added rectangular plastic boxes (MR – 16.1 × 31.8 × 45.7 cm) (Fig. 1). Shoots were pre-treated with 500 ppm IBA solution for 20 min. The leaves of shoots were not exposed to IBA solution before being placed in the MR system (Nhut et al., 2005). Details of MO and MR systems were described in Table 1.

Types of ventilation condition including one Millipore membrane with Milliseal™ (pore size 0.5 μm, Nihon Millipore Ltd., Tokyo, Japan) of diameter 2 cm.

2.2. Methods

2.2.1. Effects of AgNPs on growth and development of chrysanthemum in the MR system

Plant shoots (3 cm) were cultured in the MR system supplemented with different AgNPs concentrations (0; 2.5; 5.0; 7.5 and 10 ppm). AgNPs were added directly to the culture medium and boiled to mixes. After that, the medium was transferred into the laminar air flow hood room.

After 2 weeks in MR, 100 ml of the remaining microponic medium was collected for qualitative and quantitative quantification of microorganisms in culture media by means of Bergey, ISO 16266, NHS-F15 for bacterial culture and ISO 21527-1 (fungal classification) for fungi.

2.2.2. Combination of MR system and LEDs

Shoots were cultured in the MR system after 2 weeks of culturing under different LEDs including Green – G (565 nm), Blue – B (450 nm), Red – R (660 nm), Yellow – Y (590 nm) (Steigerwald et al., 2002), and B combined with R of different ratios (10:90, 20:80, 30:70, 40:60, 50:50 and 60:40) at 40–45 $\mu mol\ m^{-2}\ s^{-1}$. Fluorescent lamps (FL) were used as the control. All shoots were cultured at 25 ± 2 °C with humidity of 55–60% and photoperiod of 16 h/day under LEDs and FL.

2.2.3. Plant acclimatization and flowering

One thousand plants (after 2 weeks) cultured in each system (MO, MR) were transferred into greenhouse. Plantlets were planted into greenhouse containing a mixture of organic soil and sand (1:1) with a pH of 6.5, under natural light higher than 200 $\mu mol\ m^{-2} s^{-1}$ with sunshade nets, at 18–25 °C, and 80–90% relative humidity). After 2 weeks, the relative humidity was slowly reduced by gradually removing the cover. All plants were grown under natural light conditions and were irrigated with water once per day. The lighting was added from day 1 to day 30 after planting (extra 4 h per day from 8 to 12 pm by timer).

2.3. Statistical analysis

Content of chlorophyll *a* and chlorophyll *b* were determined based on maximum absorption spectrophotometer of chlorophyll *a* (662 nm) and chlorophyll *b* (645 nm) using UV-2900 spectrophotometer machine (Lichtentaler and Wellburn, 1985).

$$\text{Chlorophyll } a = (11.75\ A_{662} - 2.35\ A_{645})\ (\mu g\ g^{-1})$$

$$\text{Chlorophyll } b = (18.61\ A_{645} - 3.96\ A_{662})\ (\mu g\ g^{-1})$$

All treatments were in triplicates and each replicate included 10 culture vessels. Data were scored after 2 weeks of culturing and analysis of variance was performed. The means were compared using Duncan's multiple range Test using SPSS (Version 16.0) at p value ≤0.05 (Duncan, 1995).

3. Results and discussion

3.1. Effects of AgNPs on growth and development of chrysanthemum in MR system

After 2 weeks culturing in a MR system supplemented with different concentrations of AgNPs, results were recorded in Table 2 and Fig. 2.

Indicators of plant growth such as plant height, root length, leaf length, leaf width, fresh weight and dry weight increased proportionally with increased concentration of AgNPs added to the medium culture increased from 0 to 7.5 ppm and reached the highest at 7.5 ppm AgNPs (6.98 cm, 1.48 cm, 1.73 cm, 1.57 cm, 516 mg and 48.67 mg; respectively). The number of leaves and number of roots per plant were not significantly different among these treatments.

However, when concentrations of AgNPs in the culture medium were increased to 10 ppm, the results showed that the growth of the plants slowed down; most of the tracking parameters of the plants were lower than that of the plants cultured in 7.5 ppm AgNPs (except for the number of leaves/plant) (Table 2). In addition, 0 ppm AgNPs in the microponic medium caused the leaf to deform (Fig. 2a).

The root morphology of the chrysanthemum was different when

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